The role of translation elongation factor eEF1 subunits in neurodevelopmental disorders

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Abstract

The multi-subunit eEF1 complex plays a crucial role in *de novo* protein synthesis. The central functional component of the complex is eEF1A, which occurs as two independently encoded variants with reciprocal expression patterns: whilst eEF1A1 is widely expressed, eEF1A2 is found only in neurons and muscle. Heterozygous mutations in the gene encoding eEF1A2, *EEF1A2*, have recently been shown to cause epilepsy, autism and intellectual disability. The remaining subunits of the eEF1 complex, eEF1B, eEF1D, eEF1G and VARS, together form the GTP exchange factor for eEF1A and are ubiquitously expressed, in keeping with their housekeeping role. However, mutations in the *EEF1B2*, *EEF1D* and *VARS* genes have also now been identified as causes of neurodevelopmental disorders. In this review we describe the mutations identified so far in comparison with the degree of normal variation in each gene, and the predicted consequences of the mutations on the functions of the proteins and their isoforms. We discuss the likely effects of the mutations in the context of the role of protein synthesis in neuronal development.

Introduction

There is growing evidence that precise translational homeostasis is necessary within neurons, and that imbalances in the equilibrium of protein synthesis can result in aberrant neurodevelopment. The use of new genetic sequencing techniques in neurodevelopmental disorders (including autism, intellectual disability and epilepsy) has led to the identification of many new causative genes, either in families or in individuals with *de novo* mutations identified by sequencing parent-child trios. In recent years, individuals with mutations in genes encoding subunits of the translation elongation eEF1 complex have been discovered, implicating the elongation stage of translation as a key modulator of healthy neuronal development. Here we describe the range of mutations in components of the eEF1 translation elongation complex, all of which have been implicated in neurodevelopmental disorders. Whilst some of the mutations are seen in ubiquitously expressed factors, others affect isoforms of the protein that are preferentially expressed in neurons. Overall, the evidence demonstrates the need for tightly controlled expression of protein synthesis factors in neurons.

The eEF1 complex and functions of the subunits

Translation elongation is modulated by eukaryotic translation elongation factor 1A (eEF1A), which delivers aminoacylated tRNAs to the ribosome to lengthen nascent polypeptides. This process requires GTP; the rate of intrinsic spontaneous GDP release is low, but GDP release is increased 1000 fold in the presence of cognate guanine exchange factor eEF1B (Janssen & Möller, 1988). Together eEF1A and the subunits of eEF1B make the eEF1 complex. The complex, its architecture, composition, roles in translation and non-canonical function have been extensively reviewed in the past (Le Sourd et al., 2006; Mateyak & Kinzy, 2010; Minella, Mulner-Lorillon, Bec, Cormier, & Bellé, 1998; Sasikumar, Perez, & Kinzy, 2012).

eEF1A

eEF1A has two protein isoforms in mammals, encoded by separate genes, which are 92% identical and 98% similar at the amino acid level (Ann et al., 1991; Lund, Knudsen, Vissing, Clark, & Tommerup, 1996). Each isoform appears to function in the canonical role of translation elongation with similar protein synthesis kinetics, but with different affinities for GTP/GDP, with eEF1A1 favouring GTP and eEF1A2 showing greater affinity for GDP (Kahns et al., 1998). Despite this difference, both isoforms have been shown to bind to the eEF1B complex (Y. Cao, Portela, Janikiewicz, Doig, & Abbott, 2014; Mansilla et al., 2002).

eEF1A1 and eEF1A2 have distinct, non-overlapping expression patterns. This accounts for the requirement for both proteins, since every cell type will need some form of eEF1A to function. eEF1A1 is expressed ubiquitously throughout development but is down-regulated postnatally in neurons, cardiomyocytes and myocytes where it is replaced by eEF1A2 (Khalyfa et al., 2001; S. Lee, Stollar, & Wang, 1993). In view of their similar properties in terms of the canonical function of eEF1A in protein synthesis, it has been hypothesised that the switch between isoforms occurs because specific cell types have a requirement for distinct non-canonical functions not shared by eEF1A1 and eEF1A2 (Abbott et al., 2009). One such function is actin binding and bundling, which has been well characterised with respect to eEF1A1 (Clore, Dannenhoffer, & Larkins, 1996; Murray, Edmonds, Liu, & Condeelis, 1996). The amino acid residues known to interact with actin are found on the face of the 3D structure of eEF1A which harbours the majority of amino acid differences between the two isoforms, suggesting that eEF1A1 and eEF1A2 may have different capacities for interacting with actin (Soares, Barlow, Newbery, Porteous, & Abbott, 2009). Indeed, there is experimental evidence for differences in actin binding and bundling activities between eEF1A1 and eEF1A2 (Novosylna et al., 2017). One hypothesis, therefore, is the switch occurs so as to modify cytoskeletal interactions in neurons at different developmental stages (Abbott et al., 2009). However, it is not possible to rule out the idea that this highly conserved switch, seen in all vertebrates, occurs in response to differing needs for some aspect of protein synthesis in terminally differentiated, long lived cells.

eEF1Bα

eEF1Bα is a cognate guanine exchange factor for eEF1A (Janssen & Möller, 1988; Murakami, Ejiri, & Katsumata, 1978) operating in a magnesium dependent manner (Pittman et al., 2006). In humans, one protein coding isoform of eEF1Bα has been reported, at around 32kDa (Bec, Kerjan, Zha, & Waller, 1989), encoded by the *EEF1B2* gene. *EEF1B1* has been shown to be a processed pseudogene, whilst *EEF1B3* is a human-specific intronless gene transcribed only in brain and muscle (Chambers, Peters, & Abbott, 1998). Although eEF1Bα is essential for cell survival in yeast, with knockout strains showing growth defects (Hiraga, Suzuki, Tsuchiya, & Miyakawa, 1993), it is dispensible for short term viability, at least, of human cells in culture (see below) (Y. Cao et al., 2014). The C-terminal region of the protein encompasses the residues necessary for guanine exchange activity (Janssen & Möller, 1988), whilst the N-terminal binds to eEF1Bγ, linking eEF1Bα to the rest of the eEF1 complex (Mansilla et al., 2002). Indeed the N-terminal binding to eEF1Bγ has been shown to decrease

catalytic activity of eEF1Bα, as biochemical studies showed a 2.5 increase in GDP exchange of eEF1A2 when the N-terminal of eEF1Bα was completely truncated (Trosiuk, Shalak, Szczepanowski, Negrutskii, & El'skaya, 2016).

eEF1Bδ

eEF1Bδ is also a guanine exchange factor, sharing a high level of sequence homology at the C-terminal with eEF1Bα, where both subunits have their guanine exchange domains. (Janssen et al., 1991) (Matsumoto, Terui, Xi, Taira, & Ejiri, 1994; Morales, Cormier, Mulner-Lorillon, Poulhe, & Bellé, 1992). The sequences of the two subunits diverge at the N-terminal regions (Sanders, Raggiaschi, Morales, & Möller, 1993), where both, nevertheless, bind to eEF1Bγ (Matsumoto et al., 1994) . Three spliceforms encoded by a single gene, EEF1D, each giving rise to a protein of predicted size of about 38kDa, have been reported. The same locus also encodes a more recently described brain, spinal cord and testis specific isoform called eEF1BδL which is expressed throughout brain development in the mouse (Kaitsuka & Matsushita, 2015) (Y. Cao et al., 2014). This longer isoform includes an additional 367 amino acids at the N-terminus, a domain containing a nuclear localisation signal. Kaitsuka and colleagues demonstrated that this longer isoform, which is found in both cytoplasm and nucleus, functions during cellular stress as a transcription factor for heat shock element-containing genes. When heatshock is induced, the canonical eEF1Bδ isoform is downregulated, and eEF1BδL becomes upregulated. These changes in gene expression thus act as a two-pronged stress response system resulting in both downregulation of global protein synthesis and the concomitant upregulation of heat shock factors (Kaitsuka, Tomizawa, & Matsushita, 2011). It is as yet unclear whether the eEF1BδL longer isoform binds to the other subunits of eEF1B in the same way as the canonical isoform, and is thus able to act as a guanine exchange factor. The smaller isoforms of eEF1D are ubiquitously expressed in mouse, but are more highly expressed in brain and liver at fetal and neonatal stages than from p10 onwards (Y. Cao et al., 2014). In contrast, eEF1BδL is expressed at in brain at all developmental stages tested, increasing slightly with age (Y. Cao et al., 2014).

eEF1Bγ

eEF1Bγ is encoded by a single gene, *EEF1G*, that is transcribed ubiquitously. eEF1Bγ possesses no innate guanine exchange activity (Kinzy, Ripmaster, & Woolford, 1994) but may have the capacity to stimulate GTP exchange via eEF1Bα (Mulner-Lorillon et al., 1994). The protein contains a GST-transferase like domain at the N-terminus, a sequence often

associated with structural proteins. eEF1Bγ has also been co-purified with tubulin (Janssen & Möller, 1988), keratin (Kim, Kellner, Lee, & Coulombe, 2007) and the endoplasmic reticulum (Sanders, Brandsma, Janssen, Dijk, & Möller, 1996), consistent with a role as an anchoring subunit.

Valyl tRNA synthetase

Valyl-tRNA synthetase (ValRS) has been shown to form a stable complex with eEF1 (1–3), the only tRNA synthetase known to do so in higher eukaryotes. tRNA synthetases catalyse the aminoacylation of their corresponding tRNA, a process that is followed by the subsequent delivery of the aminoacyl-tRNA (aa-tRNA) to the ribosome by eEF1A. The consecutive nature of these steps suggests that the purpose of the interaction between ValRS and eEF1 is to enhance the catalytic activity of ValRS (1–6) and to play a role in amino acid channelling. This channeling is the process by which there is a direct transfer of the newly synthesized aa-tRNA to the ribosome via eEF1A, without mixing the intermediate substrates with the total fluid of the cell (7). Amino acid channelling, therefore, is thought to contribute to fidelity and efficiency, and thus to translational homeostasis, by coupling two stages of translation.

Table 1: Description of eEF1B subunits.

Protein Name	Gene Symbol	Synonyms	Gene locus	Variants		Function	Tissue expression	
				Refseq	Amino acid number		Ubiquitously expressed	Tissue specific
eEF1A1	*EEF1A1*	eEF1*α,*	6q14	NP_001393	462	G protein	*†	
eEF1A2	*EEF1A2*	eEF1*α,*	20q13.3	NP_001949	463			Brain (Neurons), Skeletal Muscle, Cardiac Muscle
eEF1B*α*	*EEF1B2*	eEF1β	2q33	NP_001950	225	Guanine exchange factors	*	
				NP_001032752			*	
				NP_066944			*	
eEF1B*δ*	*EEF1D*	eEF1*δ*, eEF1D,	8q24.3	NP_115754	647			Brain, Testis
				NP_001951	281		*	
				NP_001123528	252		*	
				NP_001182132	267		*	
eEF1B*γ*	*EEF1G*	eEF1*γ,*	11q12.3	NP_001395	437	Structural anchoring protein	*	
Val-RS	*VARS*	-	6p21.33	NP_006286	1264	Aminoacyl tRNA synthetase	*	

† Expressed throughout development but switched off upon terminal differentiation in neurons, skeletal muscle and cardiac muscle.

Structure and function of the eEF1 complex

Several models of the eEF1 complex have been proposed, based on different types of interaction studies in a variety of organisms (Bec, Kerjan, & Waller, 1994; Janssen, van Damme, Kriek, Amons, & Möller, 1994; Jiang, Wolfe, Warrington, & Norcum, 2005; Mansilla et al., 2002; Minella et al., 1998; Sheu & Traugh, 1999); these have been reviewed previously (Le Sourd et al., 2006; Minella et al., 1998; Sasikumar et al., 2012). There is a consensus that eEF1A binds to guanine exchange factors eEF1Bδ and eEF1Bα at the C-terminus, the site at which where GDP exchange occurs. The latter two bind to eEF1Bγ (Janssen et al., 1994; Matsumoto et al., 1994). eEF1Bγ itself possesses no GDP exchange activity (Kinzy et al., 1994), but has been shown to bind to tubulin, and the endoplasmic reticulum, suggesting that it can function as a structural anchoring protein (Sanders et al., 1996). ValRS is bound via its N-terminal to eEF1Bδ.

Cao et al (Y. Cao et al., 2014) used siRNA to examine the effect of attenuating expression of single eEF1B subunits in mammalian cells in culture. Silencing of any one of eEF1Bα, eEF1Bδ and eEF1Bγ in several cell lines resulted in small but significant reductions in cell viability and subtle shifts in cell cycle distribution but stopped short of resulting in complete lethality. No knockout experiments have been carried out for eEF1B subunits in higher organisms but it seems likely that long term ablation of any one subunit would affect growth and viability.

Mutations in eEF1 complex subunits cause neurodevelopmental abnormalities

It is of no surprise that alteration to components of the protein translation machinery would have significant effects on cellular metabolism and function, but it is only now, with the widespread use of exome sequencing, that mutations in translation elongation factors are being associated with specific clinical conditions. Neurodevelopment seems to be particularly dependent on optimal functioning of translation factors, presumably due to the requirement for local *de novo* protein synthesis at synapses in the formation of memory. All eEF1 subunits have been shown to be enriched in proteomic studies of post-synaptic densities, consistent with a role in learning and memory (Bayés et al., 2011). However, a further explanation for the association between mutations in translation factors and specific defects in neuronal function could come from the presence of mutations in neuronal-specific isoforms of otherwise ubiquitously expressed proteins. Below we review the evidence for each component of the eEF1 complex.

EEF1A2

The subunit of eEF1 most strongly associated with disorders of neurodevelopment is eEF1A2. eEF1A2 was first implicated in neurological disorders when a spontaneous 15.8kb deletion including the promoter and first exon of the gene was identified in mice displaying a severe neurodegenerative phenotype. Mice homozygous for the deletion undergo muscular and neuronal degeneration, the onset of which coincides with the down-regulation of eEF1A1 in these cell types (Khalyfa et al., 2001; Newbery et al., 2007). eEF1A2, unlike eEF1A1, is not expressed in tissues other than neurons and muscle, so it is reasonable to assume that mutations would be better tolerated than those in ubiquitously expressed eEF1 subunits.

Analysis of the EEF1A2 gene in human populations shows that it is highly constrained (table 2). Very few coding changes are seen, and none occur in more than a handful of individuals. In contrast, multiple independent heterozygous *de novo* missense mutations in *EEF1A2* have been identified in individuals with varying neurodevelopmental phenotypes including epilepsy, autism and intellectual disability (table 3). Initially, the mutations described were all associated with severe disorders, but more recently mutations in *EEF1A2* have been found in milder cases of epilepsy (Epi4K consortium & Epilepsy Phenome/Genome Project, 2017).

A homozygous mutation in *EEF1A2* has recently been described for the first time in the human population, occurring three times within a single family. Children homozygous for the P333L mutation showed failure to thrive, global developmental delay, severe epilepsy, and dilated cardiomyopathy leading to death in early childhood. Both parents were heterozygous for the same P333L missense mutation, but had no overt clinical features (the father has slight behavioural abnormalities). The P333L mutation is not seen in ExAc and is predicted to be pathogenic, but is clearly much milder in a heterozygous form than the previously described mutations, consistent with wide ranging allelic heterogeneity for mutations in this gene.

The unusual profile of mutations in *EEF1A2* in human, in which all mutations with one possible exception are missense, suggests either that heterozygous loss of function is incompatible with life, or that the missense mutations confer a gain of function or dominant negative effect. In fact, deletions encompassing the *EEF1A2* gene have been identified; in each case the affected individual has epilepsy and ID/developmental delay, but as the deletions also affect neighbouring genes such as *KCNQ2*, another well-established epilepsy gene, it is impossible to ascribe the phenotype to any one deleted gene (Faheem et al., 2015).

That these individuals survive does, however, suggest that haploinsufficiency for *EEF1A2* is not lethal and that the missense mutations may result in some form of cellular toxicity.

EEF1B2

The *EEF1B2* gene encodes the guanine exchange factor subunit eEF1Bα. A homozygous 3 kb mutation in *EEF1B2*, predicted to result in the loss of an exon, has been described in three siblings, all with moderate intellectual disability (Najmabadi et al., 2011). It initially seems unexpected that a mutation in the ubiquitously expressed guanine exchange factor for both eEF1A1 and eEF1A2 would give rise to a specific neurological phenotype (in contrast to the situation with mutations in *EEF1A2*, where the gene is expressed specifically in neurons). However, *EEF1B2* appears not to be essential for cell viability, at least in the short term (Y. Cao et al., 2014), and the mutation might not lead to complete loss of function. Furthermore, it is possible that there is some degree of functional redundancy between eEF1Bα and eEF1Bδ in terms of guanine exchange. The fact that this intellectual disability-causing mutation in a ubiquitously expressed gene apparently spares other cell types lends support to the idea that neurons are more susceptible to perturbations in protein synthesis.

EEF1D

Mutations in the *EEF1D* gene encoding the second guanine exchange factor eEF1Bδ have also been associated with neurodevelopmental abnormalities. A homozygous truncating mutation Glu24Serfs*26 has been identified in three cousins with severe intellectual disability and microcephaly (Reuter et al., 2017). This mutation is in first exon of the gene, which is found only in the neuronal- and testis- specific eEF1BδL isoform described previously (Kaitsuka & Matsushita, 2015). Figure 1 shows the position of the *EEF1D* mutation relative to the alternative isoforms.

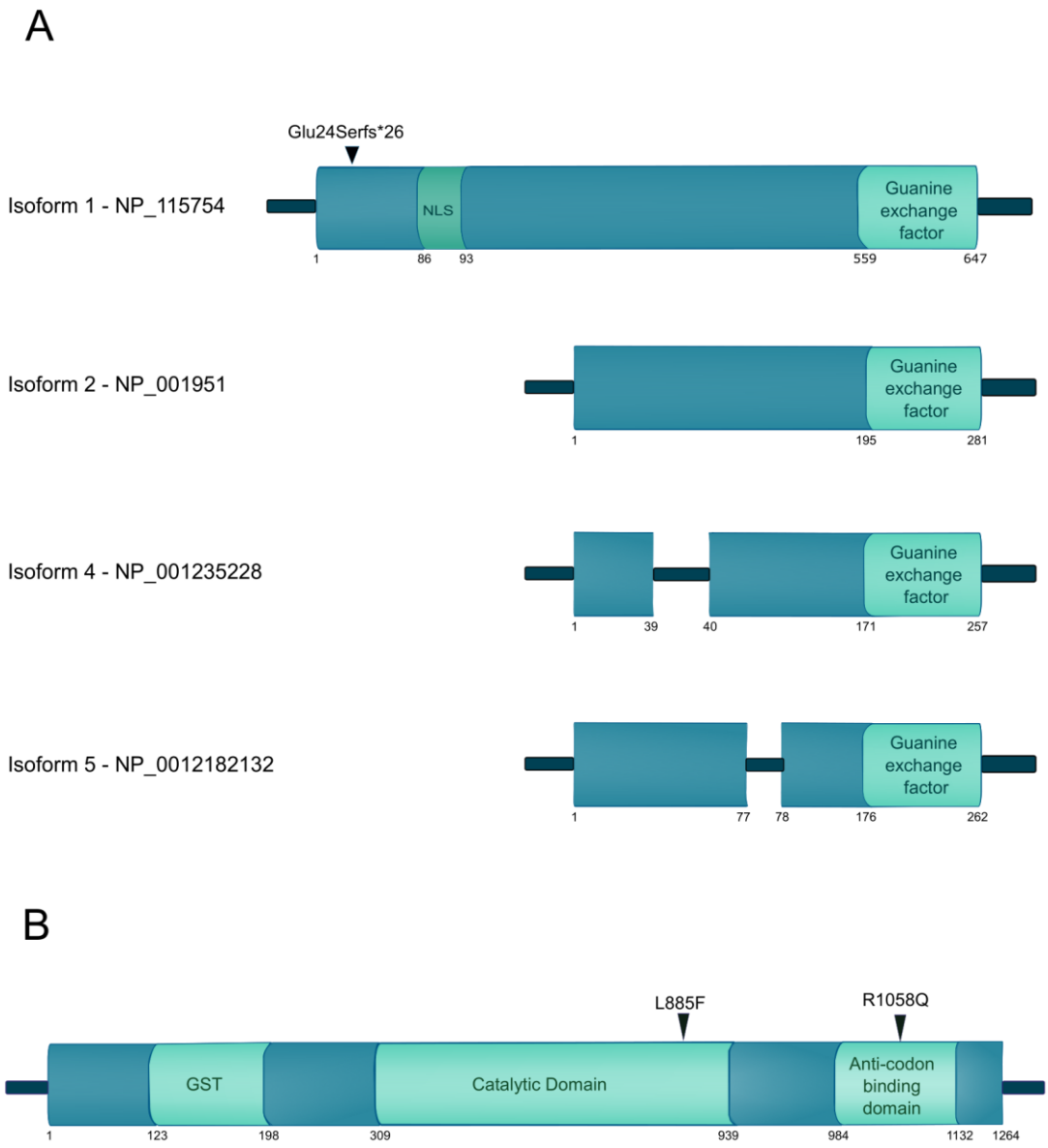

Figure 1: Schematic diagram depicting aligned protein coding sequences for the 4 isoforms of eEF1D described by Kaitsuka & Matsushita (Kaitsuka & Matsushita, 2015). Triangle marks location of eEF1D mutation identified. Mutation shown to only impact the neuronal isoform eEF1BδL isoform.

VARS

The *VARS* gene encodes valyl-tRNA synthetase (ValRS). Homozygous missense mutations (c.C2653T; p.L885F and c.G3173A; p.R1058Q) have now been found in two independent individuals, each presenting with severe ID/DD, microcephaly, seizures and cortical atrophy on MRI (Karaca et al., 2015). ValRS, along with other tRNA synthetases, has acquired non-catalytic domains during evolution. These domains do not appear to influence the canonical function of tRNA synthetases but have become evolutionarily conserved, suggesting that aminoacyl tRNA synthetase (AARS) genes may have gained additional non-canonical functions. Indeed, some splice forms are reported to be catalytic nulls, highlighting the possibility that AARS have developed functions beyond their role in protein synthesis (Lo et al., 2014). Lo and colleagues assessed tissue specific expression of these alternative splice variants, including that of splice variants encoded by the *VARS* gene, but brain was not included in the analysis. It is possible therefore that alternative splicing of *VARS* creates a neuronal specific isoform which is disproportionately affected in individuals with VARS mutations. Alternatively, the neurological phenotype seen in these individuals may again reflect a greater dependence of neurons on optimal protein synthesis.

Table 2: ExAc data for genes encoding eEF1 subunits showing extreme constraint for *EEF1A2*

HGNC symbol	Mutation	Expected no. variants	Observed no. variants	Constraint Metric
EEF1A2	Missense	211.4	29	z = 6.14
	Loss of function	10.1	0	pLI = 0.96
EEF1B2	Missense	76.4	72	z = 0.25
	Loss of function	8.3	2	pLI = 0.33
EEF1D	Missense	267.9	282	z = -0.42
	Loss of function	17.8	4	pLI = 0.39
VARS	Missense	518.5	381	z = 2.95
	Loss of function	51.8	11	pLI = 0.68

Data on mutation frequency from ExAc, the aggregation of data from 60K exomes. The Z score indicates deviation from expectation, where the higher the value the greater the constraint. pLI is the probability of loss of function tolerance; the closer the value to 1, the

lower the tolerance of the gene to LOF mutations. Lek *et al* (Lek et al., 2016) consider a pLI value of greater than 0.9 to indicate extreme intolerance, and *EEF1A2* is amongst the top few percent of most highly constrained genes.

Table 3: Mutations described in genes encoding subunits of the eEF1 complex

HGNC symbol	Protein	Mode of inheritance	Mutation (no. of affected individuals)	Phenotype	Other notes	Reference
EEF1A2	eEF1A2	Heterozygous *de novo*	G19R (1) A46S (1) G70S (4) I71L (1) D91N (1) A92T (1) D97N (1) F98L (1) E122K (4) E124K (1) D252H (2) R266W (1) N314K (1) R382H (1) E388K (1) R423C (1) T432M (1) Del 457-461 (1) V437F (1) A461V (1)	Neurodevelopmental abnormalities (epilepsy, autism and severe intellectual disability)		(de Kovel et al., 2016; de Ligt et al., 2012; Epi4K consortium & Epilepsy Phenome/Genome Project, 2017; Inui et al., 2016; Iossifov et al., 2014; Lam et al., 2016; Landrum et al., 2014; Lelieveld et al., 2016; Lopes et al., 2016; Nakajima et al., 2015; Veeramah et al., 2013)
EEF1A2	eEF1A2	Homozygous	P333L (3 siblings)	Dilated cardiomyopathy, epilepsy		(S. Cao et al., 2017)
EEF1D	eEF1Bδ	Homozygous	Truncating Glu24Serfs *26	Severe intellectual disability, microcephaly	3 siblings (consanguineous family)	(Reuter et al., 2017)
VARS	Valyl tRNA synthetase	Homozygous	L885F	Severe intellectual disability, seizures, microcephaly, cortical atrophy	2 siblings (consanguineous family)	(Karaca et al., 2015)
VARS	Valyl tRNA synthetase	Homozygous	R1058Q	Severe intellectual disability, seizures, microcephaly and cortical atrophy.	1 individual (consanguineous family)	(Karaca et al., 2015)

Why do mutations in genes with housekeeping functions lead to neurological disorders?

For each of the genes listed above, the phenotype associated with mutations is predominantly one of abnormal neurodevelopment. Given the role of eEF1 in translation, it seems at first glance surprising that all organ systems are not affected. There are two obvious possible explanations; one, each of these genes has a brain-specific isoform that is exclusively affected by the known mutations, or two, that neurons are more susceptible to perturbations in translation than are other cell types, that. In fact there is good evidence, as discussed below, to believe that each of these explanations may be true, depending on circumstance.

Neuronal- specific isoforms?

In the case of mutations in *EEF1A2*, there is a direct connection between site of expression and phenotype, as the gene is expressed only in neurons and muscle (both skeletal and cardiac). The mutational spectrum so far established, with multiple individual missense mutations occurring *de novo* and very few coding changes found in normal exomes (table 2), presents a picture of a gene very intolerant of any mutation. The severity of the neurodevelopmental phenotype seen, even in heterozygotes, is consistent with this picture. The only known cases of homozygous mutation in humans (in a single family) manifested as cardiomyopathy in addition to impaired neurodevelopment and epilepsy, but again this is consistent with the tissue-specific expression pattern of *EEF1A2*. Mice that carry biallelic mutations, either G70S missense or null, die before 4 weeks of age of seizures, sudden death, and/or neurodegeneration (Davies et al., 2017). Further mouse mutant lines will need to be established and analysed in order to establish whether cardiomyopathy is specific to the P333L mutation or whether there is a fundamental difference in the way mice and humans respond to lost or compromised eEF1A2. Nevertheless, the key point is that the restricted expression pattern is consistent with organ systems affected.

As described above, a brain and testis specific alternative splice form encoded by *EEF1D* has been identified, called eEF1BδL (Kaitsuka et al., 2011). This alternative splice form, which contains an additional exon encoding an N terminal extension of the canonical protein, acts as a transcriptional activator of heat shock genes. However, the C-terminal region of the protein is conserved between the two isoforms, suggesting that both could also function in translation elongation. The intellectual disability-causing mutation in the *EEF1D* gene is found in this additional brain- and testis-specific exon, such that any deleterious effects would be expected

to be confined to these tissues, but it is not yet clear whether the mutations impact on translation elongation, heat shock activation, or both.

Differential effects of mutations on neuronal protein synthesis?

It is well established that neurons, with their high metabolic requirement, are particularly sensitive to perturbations in protein synthesis. The synapse is a dynamic environment that needs to be able to respond quickly to stimuli in order to promote synaptic maintenance, growth and plasticity (Fernandez-Moya, Bauer, & Kiebler, 2014; Martin & Ephrussi, 2009; West & Greenberg, 2011). Furthermore, the compartmentalised structure of neurons, and their resulting need for spatiotemporal control over the synthesis of new proteins, is likely to make them more vulnerable than other cell types to mutations that affect translational control. Localised protein synthesis is essential to allow proteins that would undergo rapid turnover to reach the synapse before degradation can occur (Alvarez & Torres, 1985; Piper & Holt, 2004). Studies of mutations implicated in neurodevelopmental disorders specifically led to the "synaptic hypothesis", that neurodevelopmental disorders result from impaired assembly and/or maintenance of synapses (Gigek et al., 2015). Elongation factors have certainly been shown to be key in the lasting potentiation of synapses (Giustetto et al., 2003; Roy et al., 2018). Both isoforms of eEF1A have been reported to be present at synapses and to have a role in clustering of gephyrin, a scaffold protein involved in anchoring receptor molecules to postsynaptic membranes (Becker, Kuhse, & Kirsch, 2013). Overall, then, these findings, lend weight to the argument that mutations in even widely expressed translation elongation factors could lead specifically to synaptic or overall neuronal dysfunction, resulting in neurodevelopmental disorders.

Mutations in other components of the translational machinery have also been implicated in neurodevelopmental disorders. Fragile X syndrome, the most common inherited form of intellectual disability, results from the loss of function of a translational regulator, FMRP, and the failure to repress translation of specific mRNAs in neurons (Darnell & Klann, 2013). Mutations in amino-acyl tRNA synthetases other than VARS have been identified in numerous neurological disorders including peripheral neuropathy, epilepsy, intellectual disability and microcephaly (Coughlin et al., 2015; Kapur & Ackerman, 2018; Kapur, Monaghan, & Ackerman, 2017; Kodera et al., 2015; Musante et al., 2017; Nakayama et al., 2017; Tsai et al., 2017; Zhang et al., 2014). Unlike VARS, these other amino-acyl tRNA synthetases are not associated with the eEF1B complex, and there is no evidence that the

clinical mutations have arisen in neuronal-specific transcripts. There is thus significant evidence to support the hypothesis that mutations to key components of the translational machinery affect neurons more severely than other cell types. Alternatively, if the role of VARS in eEF1 is to support amino acid channeling, as suggested by Negrutskii et al (Negrutskii, Shalak, Kerjan, El'skaya, & Mirande, 1999), perhaps the neurodevelopmental phenotypes associated with mutations in *VARS* reflect a greater dependence of developing neurons than other cell types on channeling.

Whilst it is well established that perturbations in protein synthesis can lead to neurodegeneration (J. W. Lee et al., 2006), the disorders described in this review all affect neurodevelopment, but with differences in severity and phenotype depending on the function of the mutated gene, and the nature of the mutation in that gene. Establishing the developmental expression pattern of specific genes may shed light on the processes likely to be affected by mutations in those genes. With increasing RNAseq data being generated and collated, we can now study expression of genes across developmental timepoints. Resources such as BrainCloud and BrainSpan enable researchers to use both top-down and bottom-up approaches to identify genes which would likely be involved in neurodevelopmental disorders and the neurodevelopmental timepoints most likely to be impacted by the presence of mutations in specific genes (Kang et al., 2011; Tebbenkamp, Willsey, State, & Sestan, 2014). Whilst transcripts encoding eEF1 subunits eEF1A1, eEF1B2, eEF1D and eEF1G are expressed throughout brain development, generally declining after birth, eEF1A2 expression increases throughout embryonic development, peaking after birth in a pattern consistent with that seen for synapse development and maturation. This is consistent with data from mice and rats (Chambers et al., 1998; Khalyfa et al., 2001; S. Lee, Wolfraim, & Wang, 1993). It is possible, therefore, that mutations in the gene encoding eEF1A2, in contrast to other translation factors, may exert a greater effect on synaptogenesis than on neuronal proliferation. More detailed functional studies, including developmental timepoint specific conditional gene targeting, will shed further light on these issues, and will help us to understand not only how protein synthesis perturbation affects neurons, but why different neurodevelopmental phenotypes arise from mutations in apparently housekeeping genes.

Our knowledge of the effects of mutations in elongation factors contributes to the growing understanding of the importance of neuronal protein synthesis. Although it is well established that the disruption to the initiation phase of translation can have neurodevelopmental consequences, it has only relatively recently become clear that mutations in elongation

factors can also affect neurodevelopment. Whilst the neuronal specific phenotype is readily understandable in the cases of both *EEF1A2* and *EEF1D*, it is initially more surprising that partial deletion of the *EEF1B2* gene should specifically affect neurons. These cases now join the large number of neurodevelopmental phenotypes resulting from mutations in housekeeping genes, highlighting the requirement of neurons, in comparison to other cell types, for exquisitely controlled protein homeostasis.

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